

In silico transitions to multicellularity

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The emergence of multicellularity and developmental programs are among the major problems of evolutionary biology. Traditionally, research in this area has been based on the combination of data analysis and experimental work on one hand and theoretical approximations on the other. A third possibility is provided by computer simulation models, which allow to both simulate reality and explore alternative possibilities. These in silico models offer a powerful window to the possible and the actual by means of modeling how virtual cells and groups of cells can evolve complex interactions beyond a set of isolated entities. Here we present several examples of such models, each one illustrating the potential for artificial modeling of the transition to multicellularity.

Keywords: Major Transitions, Artificial Life, Complexity, Multicellularity

Contents

I. The physics of multicellularity	1
II. Evolving differential adhesion	3
III. Multicellularity for free	5
IV. Evolving multicellular aggregates	7
V. Physical forces and ecological scales	9
VI. Combinatorial explosions and the Cambrian conundrum	13
VII. Discussion and prospects	16

I. THE PHYSICS OF MULTICELLULARITY

The transition to multicellularity is tied to the emergence of interactions among previously isolated cells. As a problem in complexity (Anderson 1972) we could say that a multicellular system defines a level of organization whose global properties cannot be reduced to the properties of the individual units. This statement is relevant for many reasons. First, because the presence of an evolutionary innovation necessarily requires the cooperation between previously unrelated units (Schuster 1996). Once such interactions are in place, a network of connected elements needs to be considered in order to understand what is now at work. In early phases predating the transition to complex multicellular life forms, the network involved cell-cell as well as cell-substrate interactions.

In dynamical terms, the transition required the emergence of cooperation among elements, which share a common space where they relate to each other and respond to environmental changes in a concerted manner. Most theoretical and computational approaches to this problem do not take into account the fact that these systems are formed by physical objects and it might seem not so relevant when dealing with the generic mechanisms associated to cooperative dynamics. As usual, the level of detail that is used in a model scales with the type of question we wish to answer. If we search for general principles defining the appearance of cooperative aggregates, general models considering population dynamics and gene interactions are enough. But multicellularity also connects us with

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the first steps towards developmental programs and a previous step to other major innovations. Such innovations are always associated with novelties in the ways cells and tissues interact. The spatial arrangement of cells and the diversity potential provided by space and thus a explicit introduction of spatial degrees of freedom is essential.

Meaningful models of evolutionary dynamics of multicellular systems need to consider the role of generic physical mechanisms of morphogenesis that are not the result of complex regulatory processes. In this context, physical forces including gravity, adhesion or diffusion, and their generative potential, are considered (Newman and Comper 1990; Goodwin 1994). The interplay between these mechanisms allows for spontaneous pattern formation through segregation of cell types, differential cell growth and mortality. Some of these generic, pattern-forming mechanisms likely predate the early history of both pre-cellular and multicellular life forms (Forgacs and Newman 2005; Solé et al 2007; Solé 2009), along with others controlled by genetic circuits .

Using some of these mechanisms, an evolutionary model of form can be constructed. Moreover, since some of these mechanisms seem to strongly constrain the repertoire of potential structures that can be generated, they also offer a powerful framework to understand the origins of convergent designs (Alberch, 1980). In this context, as pointed out by John Tyler Bonner, simple explanations based on mathematical and computational models can help to grasp the principles of multicellular organization (Bonner 2001; Forgacs and Newman 2005). As noticed in (Newman and Bhat 2008) the interplay between physical constraints and genetic regulatory mechanisms has been traditionally overlooked in most studies, with few exceptions (see for example Eggenberger 1997; Coen et al 2004; Cummings 2006; Doursat 2008; Kaandorp et al. 2008).

Although physics and embodiment are usually discussed in the context (or at the level of) organisms or tissues, there is another level of embodiment that requires attention: the external world, whose fluctuations and properties influence the repertoire of adaptations that can be available. Thus we could add other factors playing a role in the early stages of multicellularity, including the ecological context and the physics of the environment should also be taken into account.

Here we present five examples of *in silico* models illustrating different aspects of the emergence of multicellularity. They involve different levels of complexity and address different questions, although all illustrate the generative potential of the role played by the basic physics that pervades the interactions among virtual cells.

Box 1: Modelling cell sorting under differential adhesion

The dynamics of cell sorting can be easily modelled by considering a simplified lattice model where the movement of cells is constrained by their local preferences. Specifically, let us consider a $L \times L$ lattice Ω , i. e. a discrete set of sites:

$$\Omega = \{(i, j) \in \mathbb{Z}^2 | 1 \leq i, j, \leq L\}$$

Each site $(i, j) \in \Omega$ is characterized by a "state", indicated as $S(i, j)$. This state can be 0 if the site is empty and either 1 or 2 if occupied by cells. The two possible "cell" states indicate different cell types with different adhesion properties. Cells can be more or less prone to remain together and also might tend to either avoid or attach to the external medium. Since cell-cell (and cell-medium) interactions are necessarily local, (figure 1) a given cell can only interact with a maximum of eight nearest neighbors. If $J(S(i, j), S(k, l))$ indicates the energy associated to the interactions between the sites (i, j) and (k, l) , a matrix of interaction coefficients will be defined, namely

$$\mathbf{J} = \begin{pmatrix} J(0, 0) & J(0, 1) & J(0, 2) \\ J(1, 0) & J(1, 1) & J(1, 2) \\ J(2, 0) & J(2, 1) & J(2, 2) \end{pmatrix}$$

which is obviously symmetric, i. e.

$$J(a, b) = J(b, a)$$

and

$$J(0, 0) = 0.$$

The model allows cells to move to a neighboring position by switching the two local states provided that the final state is more likely to happen, i. e. consistent with the optimization of cell preferences. This can be obtained using an energy function, defined as

$$\mathcal{H} = \sum_{i,j} \sum_{k,l} (1 - \delta_{s(i,j)s(k,l)}) J(s(i,j), s(k,l))$$

where the sum is performed only over nearest pairs. Here $\delta_{mn} = 1$ when $m = n$ and zero otherwise, and thus the term $1 - \delta_{mn}$ just discards pairs of sites with identical states.

Each step, we choose a random site, another neighboring site and compute the new energy \mathcal{H}' and compare it to the original one \mathcal{H} . If the difference

$$\Delta\mathcal{H} = \mathcal{H}' - \mathcal{H}$$

is positive, an increase in energy would occur and thus is discarded. Instead, when

$$\Delta\mathcal{H} < 0,$$

the largest the difference the more likely is the change to happen. This is a simplified model and more sophisticated approaches were developed by Glazier and co-workers, involving "cells" composed of connected sites defining a specific cell, whose shapes can change in realistic ways (Graner and Glazier 1992; Glazier and Graner 1993).

II. EVOLVING DIFFERENTIAL ADHESION

Our first example is a discrete model of evolution where digital "creatures" composed by many simplified cells interact through discrete adhesion forces. Hogeweg's model considers a population of model organisms that is evolved using a *genetic algorithm* (Mitchell 1998; Forrest 1993) that allows to search over shape space under different selection pressures (Hogeweg 2000a, 2000b). Hogeweg's approach considers the growth and development of a simulated embryo. The model

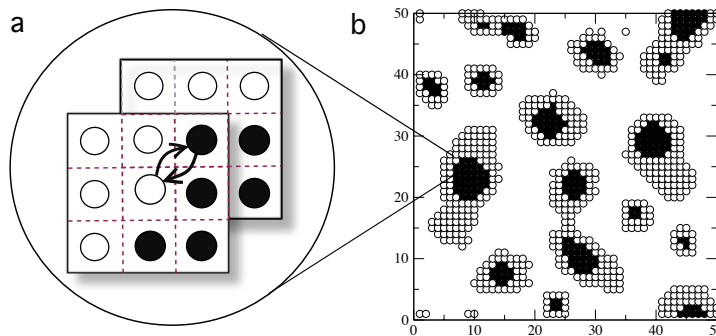


FIG. 1 Pattern formation through differential adhesion. Here a system composed by two types of cells (open and filled circles) are initially scattered at random on a two-dimensional lattice. Cells move and can flip their location with a neighboring cell (a) provided that the final energy is reduced (Box 1). Eventually, a stable arrangement of ordered cell assemblies (b) is obtained. In this example, the adhesion parameters satisfy $J(1, 2) > (J(1, 1) + J(2, 2))/2$ and $J(1, 1) > J(2, 2)$.

description includes an internal boolean gene network (see Kauffman 1993), the evolution of which leads to different adhesion among cells, cell division and death caused by stretching and compression, cell migration and differentiation.

Adhesion is introduced using very simplified but effective physical models (Graner and Glazier 1992; Glazier and Graner 1993; Sawill and Hogeweg 1997) and is one of the main players influencing the evolutionary dynamics of these virtual metazoans and their potential for diversification, consistent with the role played by development in the context of morphological radiations. Cell adhesion can easily promote the spatial organization of an initially disorganized, mixed group of cells. This is a very robust, repeatable and predictable mechanism of organization that amplifies initial disorder experienced by a mixed set of cells that move in space and aggregate with other cells under differential adhesion. The preferential adhesion mechanism is responsible for the sorting of cells in space, in a way that corresponds to the behavior of immiscible fluids (Forgacs and Newman 2005).

As pointed out by Hogeweg (2000b) differential cell adhesion is regulated by the gene network affecting cell behavior and the communication between cells through cell-cell interactions. The model considers different types of fitness functions but the only strong pressure is directed to maximizing the diversity of cell types and thus there is no explicit search for special spatial arrangements or predefined developmental programs. Hogeweg's work revealed the existence of a neutral landscape of possible phenotypes that pervades the punctuated nature of transitions (Hogeweg 2000b; see also Fontana and Schuster 1998). Long periods of stasis are characterized by slow increases in fitness as small variations in phenotype are achieved. Selection for diverse gene expression patterns is used (see also Solé et al. 2003), a choice that can be justified by an observed, well-known trend. The number of cell types is a good measure of complexity, which is known to increase through metazoan evolution (Carroll 2005). Increases in cell type number provide a high potential for further evolution of anatomical and functional complexity, essentially through division of labor and the formation of specialized tissues (Maynard-Smith and Szathmáry 1995).

Since the imposed selection pressure is rather generic, no special constraints are posed on the way genes interact and influence cell arrangements; no particular, predefined architectures and developmental plans are favored. The model is able to evolve complex forms, and in the process of evolving them, different remarkable changes take place. Complex shapes and some familiar ways of obtaining them (such as tissue engulfing, budding, etc) appeared and complex interactions between apoptosis or migration emerged. As pointed out in Hogeweg (2000a), morphogenesis itself emerges

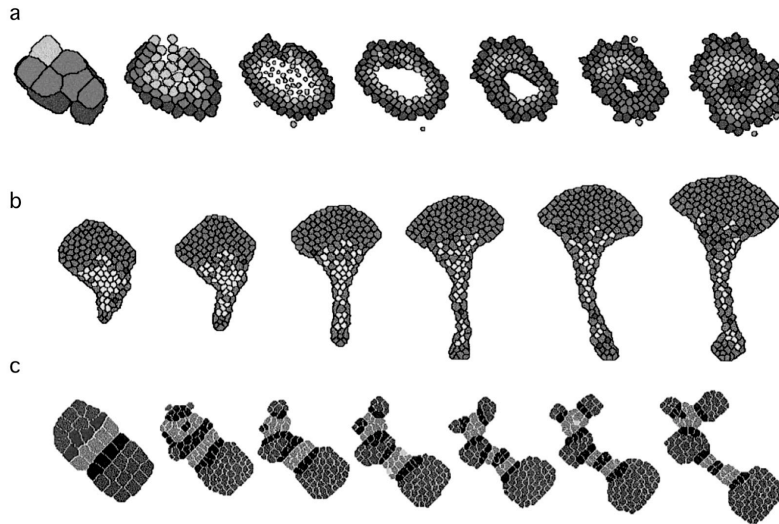


FIG. 2 Some examples of the developmental programs obtained in Hogeweg's *in silico* experiments involving the evolution of cell adhesion and differentiation (see Hogeweg 2000a). Differential cell adhesion leads to cell migration and tissue remodeling, including intercalation of cells and cell layers. Here we show three different outcomes of the evolution process, where selection for maximal cell differentiation produces, as a side effect, morphogenetic processes and pattern formation. Among the developmental processes observable, we find: (a) cell migration and engulfing, (b) budding and elongation and (c) cell death and re-differentiation.

as a byproduct of optimization for cell diversity. It is worth noting that other works involving cell type richness as a fitness function favor the explosion of pattern forming motifs as soon as a threshold of genetic complexity is reached (Solé et al. 2003).

III. MULTICELLULARITY FOR FREE

One of the most relevant and striking features of the transition to multicellularity is the fact that it took place multiple times in different lineages in the history of evolution (Abedin 2010; King 2004; Bonner 2001; Knoll 2011; Niklas and Newman 2013). For several authors this suggests that there is a certain component of inevitability in this process (Buss 1984; Grossberg and Strahmann 2007), that cooperation and specialization are such powerful innovations that convergent evolution into multicellularity ensued. In this vein, if Hogeweg’s model proves that selecting for cell diversification can lead to co-select unexpected, emergent properties and behavior, this second model by Kaneko and colleagues (Kaneko and Yomo 1998) shows that even when there is no selection at work, cells can easily drift into multicellular phenotypes including differentiation and spatial patterning.

As in the last example, the model starts with a single cell embryo that can develop into simple yet hallmark structures (see figure 4), just by allowing cell-cell and cell-environment interactions. The former are simulated by non-preferential adhesion between close cells and the latter by transport and processing of chemical species present in the virtual environment, which cells use to grow and divide. The internal workings of the cells are modeled by sets of coupled ordinary differential equations which describe autoregulatory transcription factor networks (see box 2). Remarkably, Kaneko's work shows that even if the internal networks, the initial state of the cell and the environment composition are randomized, a significant fraction of all possible cases develops consistently into virtual organisms with life-like features.

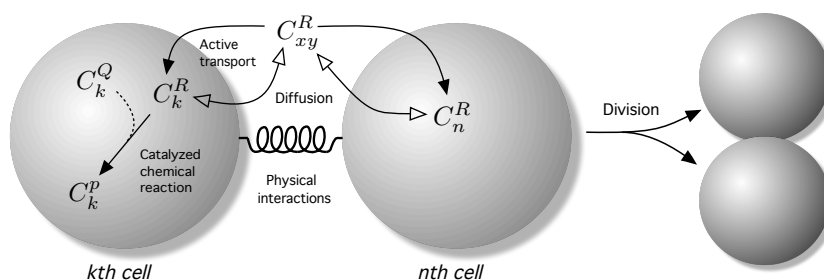


FIG. 3 Schematic representation of Kaneko’s model, depicting the internal cell dynamics (catalyzed chemical reactions governed by sets of differential equations) as well as cell-cell interactions (diffusion and active transport of metabolites). The accumulation of key species up to a fixed threshold leads to division and stochastic allocation of the molecules between the new daughter cells. Redrawn from (Kaneko and Yomo 1999).

Box 2: Dynamical networks of differentiation

Internal dynamics Cellular states are defined by the set of concentration of each species, namely $C_k^1(t), \dots, C_k^l(t)$ for the concentration of the l species in the k th cell at time t . A reaction matrix with notation $W(R, P, Q)$ is constructed, each position taking the value 1 if there exists a reaction from chemical R to chemical P catalyzed by Q and 0 otherwise. The reaction matrix is generated randomly before each simulation and remains fixed during that simulation. Concentration change in this simple set of 3 species, namely Reactant R , Product P and Catalyzer Q . The equations read:

$$\frac{\partial C_k^P}{\partial t} = W(R, P, Q) C_k^R (C_k^Q)^2$$

$$\frac{\partial C_k^R}{\partial t} = -W(R, P, Q) C_k^R (C_k^Q)^2$$

Cell-Environment dynamics The environment is simulated by a lattice of equal sized patches, each one of them characterized by $C^1(x, y, t), \dots, C^l(x, y, t)$. Two different modes of material transfer are considered, passive diffusion and active transport. Diffusion takes place among neighbor finite elements and between occupied finite elements and the cells in them. It is assumed proportional to the difference of concentrations. Active transport, on the other hand, is considered to be only proportional to the concentration in the local finite element and modulated by the internal state of the cell. Thus the term accounting for diffusion and transport of the i th chemical in the k th cell is:

$$\frac{\partial C_k^i}{\partial t} = D_e \nabla^2 C^i(x, y) + D_c \sigma_i (C^i(x, y) - C_k^i) + T_c C^i(x, y) \sum_{j=1}^l C_k^j$$

Where D_e and D_c stand for environment and cell diffusion coefficients respectively. The term σ_i represents the membrane permeability to the i th chemical and, as a simplification, can only take two values (0 or 1), fixed at random before the simulation. T_c is the transport constant equal for all species and cells. The boundary conditions of the system are maintained to a high concentration of a source metabolite, which can be interpreted as feeding the tank where the simulated cells are placed with raw materials.

Cell division and death Division is considered to be the direct consequence of the accumulation of key, predefined chemicals inside the cell. A threshold function is defined, namely

$$\int_{t_0}^{t_0+\tau} C_k^l dt \geq D$$

as the criterion for cell division and splitting of resources between daughter cells, which are generated close to the position of the mother cell. Likewise, if the threshold condition

$$\sum_i C_k^i < S$$

is met, cells are removed from the simulation.

Kaneko and colleagues observed that from the subset of configurations displaying cell growth and intermediate connectivity (case study in Kaneko and Yomo 1999 contains 9 paths for a system of 32 chemical species), most led to periodic or quasi-periodic changes in metabolite concentrations inside the cells, analogous to natural cell cycles observed in both unicellular and multicellular organisms. Beyond a certain population threshold, synchronization and phase stabilization appeared among the cells in the same cluster, the first steps towards cooperation and collective action in cell societies. Further increase in the numbers within the ensemble enables the divergence of the mean chemical concentrations and the cycle itself (i.e. the differentiation of the cells). These new trajectories in the phase diagram were stabilized by mutually reinforcing metabolite exchanges between neighboring cells.

This process of generation of variants from previously equal individuals, dubbed isologous diversification, provides a solid testbed to study both the community effect (Carnac and Gurdon 1997) and positional information (Wolpert 1969) theories. Moreover, after undergoing a first round of differentiation, cells could subsequently change into previously unavailable cell types, thus creating a tree-like hierarchical structure observed in natural developmental lineages. Some of this virtual organisms displayed other key features of living systems like robustness to noise during development (Kaneko and Yomo 1999), resistance to "injury" through regeneration of spatial patterning

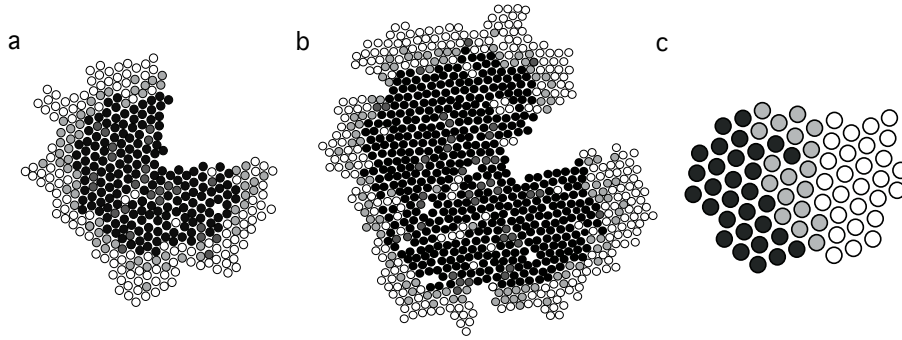


FIG. 4 Some of the multicellular organisms obtained by Kaneko's model (redrawn from Furusawa and Kaneko 1998; Furusawa and Kaneko 2003), the different shades of grey represent different cell types determined by mean metabolite concentration. Here we show the evolution of an organism after the removal of a quarter of the cluster (a), which leads to the regeneration of the previous ring pattern by means of differential cell growth and differentiation (b). Other natural occurring developmental forms are also observed, such as stripes (c).

(Furusawa and Kaneko 1998) and even life cycles exhibiting senile/proliferative stages (Furusawa and Kaneko 1998).

In conclusion, Kaneko's model demonstrates that even in the absence of evolution or selection, valid unicellular genotypes have the potential to create complex, emergent phenomena given that size thresholds can be surpassed. Whether this phenotypical changes suppose an increase in fitness is not of relevance here, but the feasibility to become multicellular and display potential task allocation as a side effect of cell-cell interactions. This results articulate a clear connection to Kauffman's work (Kauffman 1993) and the realization that some of the high order features observed in natural systems can arise not as a result of natural selection but the unavoidable properties of systems with high epistatic connectivity.

IV. EVOLVING MULTICELLULAR AGGREGATES

Multicellularity has been a recurrent novelty in the story of life and some clues to its origins can be found (at least at the functional level) in living unicellular systems, such as bacteria or yeast. Many unicellular species display multicellular traits (Shapiro 1998; Bonner 2001) associated to the presence of signals that provide the source of coherent population responses. As a consequence of these responses (mainly to stress signals) multicellular aggregates can form and display some degree of specialization and/or differentiation. Following these observations, a very promising approach to study the feasibility of the transition to multicellularity is the use of artificial selection in natural systems.

This strategy was put forward in a recent set of experiments (Ratcliff et al. 2012), in which

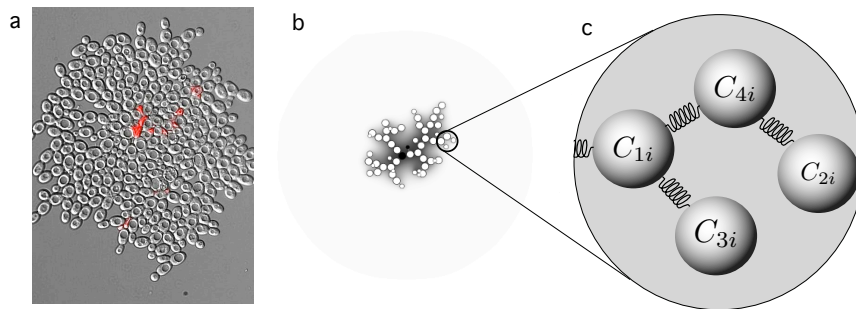


FIG. 5 The path from experimental results to in silico modeling. In (a) clusters of snowflake yeast with some apoptotic cells dyed in red in the center (from Ratcliff et al. 2012), breackage of a large cluster which generates juvenile propagules during the course of the simulation (b) and schematic representation of cell physics in the model (c) as described in box 3 (from Duran-Nebreda and Solé 2014).

the authors sequentially subcultured *S. cerevisiae* cells with the fastest sedimentation in order to force the selection of cooperating aggregates. Yeast is a specially interesting candidate to explore the potential first steps due to the fact that it already presents some pre-adaptations thought to be relevant in this major transition in evolution (Maynard-Smith and Szathmary 1995; Szathmary 1994) and its biology and multicellular states are enough well described so that new emergent phenomena can be easily distinguished from them. Remarkably, after just 60 selection events -in a timescale much shorter than previously thought- the so called snowflake phenotype appeared consistently in all cultures. These are roughly spherical clusters of cells formed not by aggregation but by defective separation of cells after division.

The clonal formation of the clusters ensures limited conflict of interest among the elements of the new multicellular individual, as discussed elsewhere (see Grossberg and Strahmann 2007; Bonner 2001; Michod 2000). Later on, the authors studied the role played by cellular fate in cluster reproduction. It was found that clusters did not reproduce through events associated to single cells but instead involved a group-level set of events that lead to the generation of a propagule. This was achieved through a division of labor in the form of the active control of apoptosis, which caused the asymmetrical splitting of the cluster once it reached a threshold size.

In order to test alternative explanations to some of the phenomena observed -particularly the presence of a group-level reproduction and its relation to division of labor- and test other potential scenarios for the rise of multicellular ensembles, a simple embodied computational model was created (Duran-Nebreda and Sole 2014). In it yeast cells are modeled as point elements in a bidimensional lattice which can fail to separate correctly after division, thus remaining attached by a spring. Cell's movement is modeled by a biased random-walk. No explicit genetic network is implemented, instead cells inherit the mother's parameters with small deviations. Two causes of cell death are tested, apoptosis as well as a simpler alternative based on the depletion of resources.

Box 3: Evolving multicellularity under size-dependent selection

Cell death caused by nutrient depletion To take into account this process, cells are placed in a bidimensional lattice that holds nutrient. Cells have an energy value M_{ni} , a division threshold M_{ni}^c , a counter on the number of divisions Δ_{ni} and an attachment probability to daughter cells once they divide p_{ni} . Nutrient concentration change in the finite element R_{ij} is given by the following equation:

$$\frac{\partial R_{ij}}{\partial t} = D \nabla^2 R_{ij} - \rho \Theta_{ij} R_{ij}$$

The Heaviside function Θ_{ij} is used to indicate the presence or absence of cells in that particular patch of the lattice (so $\Theta_{ij} = 1$ if a cell is present and zero otherwise). The diffusion operator $\nabla^2 R_{ij}$ is numerically computed following a standard discretization form:

$$\nabla^2 R_{ij} = \frac{\partial^2 R_{ij}}{\partial x^2} + \frac{\partial^2 R_{ij}}{\partial y^2} \approx \left[R_{ij} - \frac{1}{4} \sum_{kl} R_{kl} \right]$$

where D accounts for the coefficient diffusion and ρ the intake of nutrients from the culture medium. The energy change for C_{ij} is:

$$\frac{\partial M_{ij}}{\partial t} = \rho R_{ij} - \beta_c M_{ij} (1 + \kappa \Delta_{ni})$$

Where β_c represents the maintenance costs. If the energy value of a particular cell reaches its division threshold, a new cell is created and the original energy value is split asymmetrically between the cells. Conversely, cells die if: $M_{ni} \leq \delta_c$, Where δ_c is the energy limit cells can withstand.

The conclusions extracted from the simulations draw a slightly different picture on the possible first steps towards multicellularity. In it, physical constraints previously linked to a decrease in fitness for the aggregates -namely the added difficulty to attain enough nutrients to survive caused

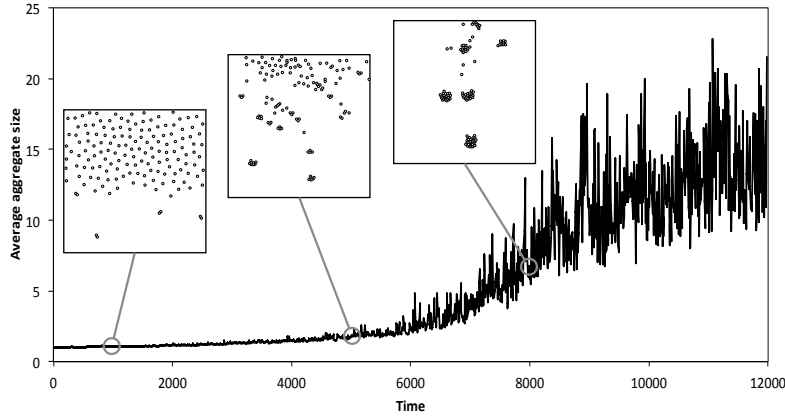


FIG. 6 *In silico* evolution of aggregates under the nutrient depletion model (Duran-Nebreda and Solé 2014). Average cluster size increases over time and stabilizes after 10^4 simulation steps (mean for 10 replicate experiments). The maximum aggregate size is dependent on the nutrient concentration fixed at the beginning of the simulation (data not shown).

by limited diffusion in the core- are shown to work in favor of reproductive fitness, debilitating the cluster structure and promoting splitting after a certain size threshold is achieved.

V. PHYSICAL FORCES AND ECOLOGICAL SCALES

An important component of a consistent theory of multicellularity, particularly in relation to the emergence of not just cooperation, but also developmental programs, requires considering community ecology in embodied models. Such models introduce physics and spatial interactions, and they naturally incorporate some selection forces, since the explicit physics introduce strong constraints to the potential forms and multicellular aggregates that can be obtained. If cell aggregates explicitly move, adhere to substrates, develop cooperation through cell-cell shared nutrients and resist external fluctuations, the evolution of adhesion and other variable features will occur under well-defined selection pressures. If these changes take place in a physical environment where available resources spread and are consumed, ecosystem-level processes might take place, some particular structures, such as budding, coherent multicellular shapes, differentiated aggregates or even life cycles could emerge. Moreover, potential changes in grazing efficiencies and the rise of predators can trigger arm races tied to changes in developmental programs, as it is likely the case for the transition between the Ediacaran and Cambrian biotas (Marshall 2006; Fedonkin 2007; Erwin and Valentine 2013).

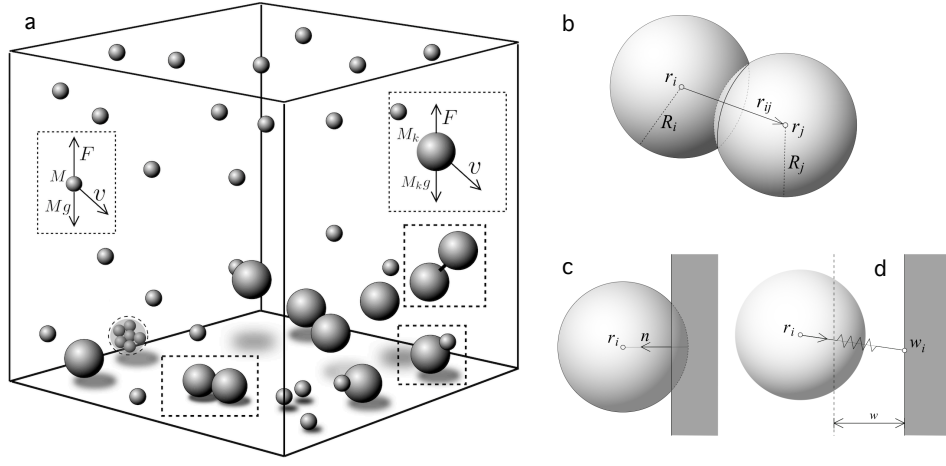


FIG. 7 Basic scheme of the components of the CHIMERA model (Solé and Valverde 2013). The system (a) is confined within a rigid cube. Nutrient particles fall from the top layer experiencing physical forces. Cells also experience the same forces, as described by Newton's laws (see Box 4). Additionally, both cells and particles get degraded. Cells can interact with the boundaries of the system as well as between them. Cells increase in mass every time they collide with a nutrient particle if they have the right internal metabolism. We also display the mechanical forces acting between cells (b) and the interactions between cells and the boundaries (c-d). In both cases, adhesion forces stabilize interactions within some range, but interpenetration is forbidden.

Box 4: Modeling ecology, physics and evolution

The CHIMERA model was build as an advanced framework introducing artificial cells as particles in a physical world where Newtonian forces, along with selection pressures, genes and metabolism are taken into account.

Cells and particles Our starting point is a population of single-cell organisms, where each cell in the initial population is identical. Cells and particles are simulated with rigid bodies moving within a fluid-like environment. A cell (particle) has spherical geometry with radius R_i , mass M_i , spatial position \mathbf{r}_i and velocity

$$\mathbf{v}_i = d\mathbf{r}_i/dt.$$

The motion of a cell is described by the standard second law:

$$M_i \frac{d\mathbf{v}_i}{dt} = \mathbf{F}_i$$

Cell movement is obtained by numerical integration of the Newton equations. Cell velocity at time $t + \Delta t$ is thus:

$$\mathbf{v}_i(t + \Delta t) = \mathbf{v}_i(t) + \mathbf{F}_i/M_i \Delta t$$

where Δt is the size of the integration step, and the total force acting on M_i will be:

$$\mathbf{F}_i = \mathbf{F}_e + \mathbf{F}_i^c + \mathbf{F}_i^n + \mathbf{F}_i^l - k_d \mathbf{v}_i + M_i \mathbf{g}$$

applied to any cell is the sum of environmental forces \mathbf{F}_e , the gravitational field \mathbf{g} , the collision force \mathbf{F}_i^c , the cell-wall adhesion \mathbf{F}_i^n and the cell-cell adhesion \mathbf{F}_i^l term, along with the drag force associated to the medium, i. e. $-k_d \mathbf{v}_i$.

Cell-substrate adhesion Attachment of cells to surfaces may provide a favorable micro-environment for cell aggregates to develop. If $D(\mathbf{r}_i)$ indicates the cell-to-wall distance, when a cell with adhesion strength to the substrate $J_i^f > 0$ is closer than the adhesion range $\delta_w > 0$, i.e.,

$$D(\mathbf{x}_i) < \delta_w,$$

we attach a spring connecting the cell \mathbf{x}_i with its projection on the wall \mathbf{x}_i^w (Figure 6). Now, the wall spring exerts the following attraction force:

$$\mathbf{F}_i^n = -k_s (||\mathbf{r}_i - \mathbf{r}_i^w|| - d_s) \frac{\mathbf{r}_i - \mathbf{r}_i^w}{||\mathbf{r}_i - \mathbf{r}_i^w||}$$

where d_s is the spring equilibrium distance, k_s is the spring constant and $\mathbf{F}_i^n = 0$ when the cell is not attached to any spring. Existing cell-wall springs can be removed with certain probability $1 - q(i)$ or when the spring length is above the maximal length, i.e.,

$$||\mathbf{r}_i - \mathbf{r}_i^w|| > d_s^m.$$

As we will see, cells can evolve cell-wall adhesion J_i^f in order to maximize the intake of nutrient particles.

Cell-cell adhesion Cells can form aggregates by attaching to other cells. Each cell has an intrinsic probability J_i^c to create a new adhesion link. Given two close cells located at \mathbf{r}_i and \mathbf{r}_j , we will set an adhesion string connecting them with probability $(J_i^c + J_j^c)/2$. The adhesion force to any cell is the sum of forces contributed by all the active cell-cell adhesion springs:

$$\mathbf{F}_i^l = - \sum_j k_l (||\mathbf{r}_i - \mathbf{r}_j|| - d_l) \frac{\mathbf{r}_{ij}}{||\mathbf{r}_{ij}||}$$

where $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$, d_l is the spring equilibrium distance, and k_l is the spring constant. Adhesion springs break spontaneously with rate $\delta \approx 0.001$ or when the spring is very large, i.e.,

$$||\mathbf{r}_i - \mathbf{r}_j|| > d_l.$$

In order to incorporate all these components, we need to build a complex simulation framework able to capture the essential physics, the presence of a population of interacting artificial agents and mechanisms of evolving the parameters that weight different metabolic and adhesion properties. Such type of model belongs to the tradition of so called artificial life approaches (Ray 1991; Langton 1991, 1995; Sipper 1995; Adami 1998) which involve the study of artificial life-like systems in artificial environments (along with a wet version associated with the construction of living systems using genetic engineering techniques). Computational models, which can reproduce realistic scenarios or completely ignore them, provide an ideal framework to explore the generative potential of an evolving set of rules allowing structures to emerge through time. Following this view, an embodied model of evolution, the so called CHIMERA model (Solé and Valverde 2013) was introduced as a way of including newtonian physics, fluctuations, evolution and ecology in a unified fashion.

The model was intended as an approach to the pre-Mendelian universe, which can be approached by studying the interplay between physical forces such as gravity, diffusion and adhesion and generic pattern-forming mechanisms. In its simplest version (Solé and Valverde 2013) Chimera considers a cubic world involving a fluid-like medium with gravity and turbulence (figure 7) where an initial set of identical "cells" exploit one of a number of potential energy particles falling from the upper side of the cube. A set of rules is then used to evolve the system:

1. Movement: both particles and cells experience both a gravitational and a fluctuating velocity field (the later associated to turbulence). Particles are removed from the system with some probability.
2. Each cell carries a given set of internal parameters and variables: they have a given size and

mass and they have a list of possible particle types that they can take and the efficiency of the grazing for each particle type.

3. Cells can attach to the surface of the walls with some probability. When they attach, a spring is used to properly define the physical interaction. Another adhesion probability is used for cell-cell adhesion. At the beginning all are set to zero.
4. If a cell interacts (collides) with a given particle, it ingests it if the efficiency for metabolizing that type of particle is non-zero. If taken, the mass of the particle gets transformed into mass of the cell.
5. Once a maximum cell size is reached, the cell splits into two daughter cells. Moreover, if the cell goes below a minimal value, it dies and it disintegrate. Detritus particles are also allowed to be part of the nutrient intake of cells.
6. Each time a cell divides, mutations can occur in the daughter cell. Metabolic efficiencies and adhesion rates can change.

The model is able to display complex forms of pre-multicellularity in terms of loose aggregates of cooperating cells. These aggregates evolve adhesion rates, both between cells but especially in relation to the physical substrate. Two major trends take place here. From the point of view of grazing efficiency, individual cells tend to become generalists: their metabolism changes in order to exploit all types of nutrient particles, although at the price of being less efficient. Secondly, in order to gather more particles, there is an advantage in providing a larger cell surface, which is possible provided that cells increase their attachment probability (adhesion force) and occupy available space on the lateral walls. This tendency, once started, is rapidly amplified and we can see (figure 8a) that eventually the cells "discover" the source of energy and occupy it. This is done by an increase of cell-floor adhesion but also increasing cell-cell adhesion and (when fluctuations are large) cooperation.

Eventually, the flow of particles is almost shut down except for cell mortality and the sedimentation of cells. Cell death creates detritus particles, which are also exploited with low efficiency. But once a critical amount of detritus gets accumulated at the bottom, a new community of specialized detritivores emerges. This new ecosystem with two layers is stable over time and represents a dramatic example of how ecosystem engineering emerges: the feedbacks between organisms and environment trigger a control of the later by the first, with a deep reorganization of energy flows (Jones et al 1994, 1997; Hastings 2006; Erwin 2008; Erwin and Tweedt 2012).

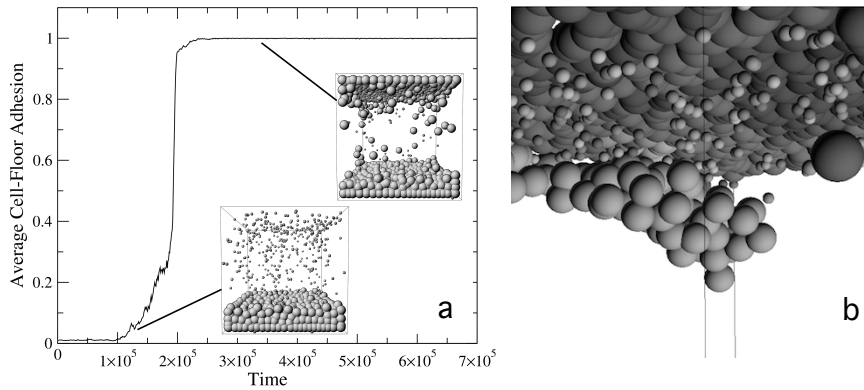


FIG. 8 (a) in the basic, newtonian CHIMERA model, the evolution of the system under enough nutrient levels drives the population from a few layers placed at the bottom to an inverted system where cells occupy and adhere to the upper wall. This occurs thanks to a broad distribution of efficiencies (i. e. evolved generalists) exploiting all resources, together with an increased adhesion between cells and specially between cells and surfaces. The main plot shows a fast growth in cell-floor adhesion towards its maximum value. Two snapshots of the system are also shown, before and after the transition. In (b) an example of an evolved multicellular aggregate is shown. This was obtained by using a more detailed implementation of the cell-cell interactions that allow aggregates to emerge Solé and Valverde 2013).

The outcome of the simulation reveals how a microscopic process of evolution associated with cell adhesion, combined with a community adaptation to the environment leads to a major innovation. Using simpler physics (Palsson and Othmer 2000; Palsson 2001; Ericson 2005; Sandersius and Newman 2008) where cells have volumes but their behavior is closer to point particles does not allow the formation of complex aggregates (Box 4). However, a more sophisticated and realistic definition of forces and spatial interactions allows the formation of multicellular aggregates (figure 8b) thus suggesting that much more can be obtained even under these simplified scenarios (Solé and Valverde 2013).

VI. COMBINATORIAL EXPLOSIONS AND THE CAMBRIAN CONUNDRUM

As a final example, we consider the problem of how complex and diverse spatial patterns of gene expression (and thus cell types) can emerge as a consequence of gene networks in development. Specifically, we consider an abstract model of pattern formation where a one-dimensional digital organism is composed of C cells each carrying the same gene network.

Box 5: Gene networks in development

A simple, discrete model of pattern formation can be implemented by defining a set of $N = G + H$ genes interacting through a one-dimensional domain involving C cells (Solé et al., 2003). Here gene states will be Boolean: genes are either active (1) or inactive (0). G genes interacting within the cell, whose state at a given time t will be indicated as $g_i^j(t)$, where $i = 1, \dots, G$ is gene number and $j = 1, \dots, C$ is cell number (ordered from anterior to posterior). The second group are generically labeled microhormones and actually involve (implicitly) some local mediators communicating neighboring cells.

The state of these H hormones will be indicated as $h_i^j(t)$. Hormones can receive inputs from any of the first G units, but they can only make output to genes in other cells. Two matrices will be required in order to define the whole spectrum of links between different elements. These two matrices will be indicated by $\mathbf{W} = (W_{ik})$ and $\mathbf{C} = (C_{ik})$, defining interactions among the G genes and between genes and hormones, respectively.

The basic set of equations of our gene network model read:

$$g_i^j(t+1) = \Phi \left[\sum_{l=1}^G W_{il} g_l^j(t) + \sum_{k=1}^H C_{ik} \delta \left(h_k^{j+1}(t), h_k^{j-1}(t) \right) \right]$$

where $\delta(x, y) = 1$ if both $x = y = 1$ and zero otherwise (i.e. an “OR” function). Similarly, hormones receive inputs only from inside the cell,

$$h_i^j(t+1) = \Phi \left[\sum_{l=1}^G W_{il} g_l^j(t) \right]$$

with additional, specific equations for the boundaries. The function $\Phi(x)$ is a threshold function, i. e. $\Phi(x) = 1$ if $x > 0$ and zero (inactive) otherwise. Specific equations are also defined for the two ends of the system.

Finally, we need to define an initial condition. The simplest choice that can be defined involves the activation of a single gene at the anterior ($j = 1$) end of the digital organism. Specifically, we set $g_i^j(0) = h_i^j(0) = 0$ for all $j = 1, \dots, N_c$, except $g_1^1(0) = 1$. These choices corresponds to maternal signals confined to the chosen end. Such initial change will propagate to the rest of the tissue provided that the network allows it.

The gene network includes both gene-gene interactions within the cell and between cells. In other words, we take into account regulatory interactions taking place within each cell together with cell communication through given signals (to be called hormones). These models have been extensively used since the early days of theoretical biology (Kauffman 1993) and provide a

simple way of approaching the problem of defining cell types and thus multicellular assemblies.

In its simplest form, we can define a gene network in terms of a set of n genes whose states g_i are confined to two possibilities, namely $g_i \in \{0, 1\}$. This binary approximation implies that genes are ON-OFF elements, which of course is a simplified picture. If the effect of gene g_i on gene g_j is given by a weight W_{ij} . If the gene interaction describes an activation or inhibition, W_{ij} will be positive or negative, respectively. No interaction is given by $W_{ij} = 0$. For simplicity, a discrete space of weights is used, namely $W_{ij} \in \{-1, 0, +1\}$. In this way, a full exploration of the potential set of states can be performed.

The state of a gene will change as a consequence of its interactions with other genes. This state is updated in discrete time units following:

$$g_i^j(t+1) = \Phi \left[\sum_{l=1}^n W_{il} g_l^j(t) \right]$$

which essentially tells us that the gene will become active or inactive if the global input acting on it is positive or negative, respectively. These networks can generate extremely simple (say, all genes inactive or active) or very complex dynamics (when chaotic changes occur). But for some ranges of connectivities, it leads to a rich diversity of stable states. If a cell type T is identified as a string \mathbf{S}_T of active and inactive genes, namely $\mathbf{S}_T = (g_1, \dots, g_n)$ (Kauffman 1993) we have a potential of 2^n alternative strings. We can expand this formalism to take into account space, if multiple cells are also taken into account (Box 5). As shown in figure 9a, this is easily implemented and a spatial pattern can be described, for each gene, in terms of its expression level (0 or 1) in different cells. A detailed analysis of this type of model reveals that some types of patterns are easily found (Solé et al 2003; Munteanu and Solé 2008; Tusscher and Hogeweg 2011). This is the case of regular stripe patterns, which is a rather common one.

What is the generative potential of a given gene network complexity level? More precisely, if we start from a small network with $G = 1$ genes and $H = 1$ hormones and expand its number, what are the consequences? These questions are relevant when we consider the early events that shaped the gene networks affecting developmental processes in the history of multicellular life. Once again, thinking in the Cambrian explosion of life, several factors can be considered (Marshall 2006; Erwin and Valentine 2013). These include external, abiotic factors as well as internal ones and they are likely to interact among them. But it would be interesting to know, even under some basic abstract model, if some particular elements can play a key role in promoting sudden changes in the amount of achievable morphologies. In order to analyze this problem, we use the presence of a genotype-phenotype mapping Ω , namely

$$\Omega : \mathbf{W} \longrightarrow \{\Phi\}$$

between the set of matrices \mathbf{W} and the corresponding set of patterns $\{\Phi\}$. In other words, for a given network W_a , the arrangement of ON and OFF genes defining a stable pattern P_a^* can be written as $P_a^* = \Phi(W_a) \in \{\Phi\}$.

Using our basic model, it is possible to explore the space of potential phenotypes through single mutations in the genotype, as defined by the matrix of gene-gene interactions. Each step in the simulation, we evolve the system by changing single elements in the matrix. Patterns that are not stable are discarded and a change in the matrix is accepted if the diversity of cells within the organism is increased (or at least remains the same). This movement in sequence space is known as an *adaptive walk* (Kauffman and Levin 1987).

Pattern-forming gene networks display sharp thresholds affecting their combinatorial potential. For small numbers of elements, i. e. when $H + G < 4$ and $H, G \leq 2$ the range of possible spatial patterns is rather limited (figure 9b) but once the critical number $H = G = 2$ is reached, all patterns become accessible (Solé et al. 2003). This is a very interesting finding, since it provides a possible logical explanation for the rapid diversification of developmental paths when genetic complexity thresholds are crossed. Along with other influences, a small increase in regulatory complexity can account for a sudden jump in the achievable diversity of developmental pathways.

Although these results are obtained from a toy model of regulatory interactions and ignore other pattern-forming factors, such as tissue organization, morpho-dynamic processes or cell division, sorting and apoptosis, the basic conclusions are likely to be robust: a relatively small increase in underlying genomic complexity can lead to rich morphogenetic potential (Marshall 2006). In earlier models of evolution on fitness landscapes (Niklas 1994) high diversity is linked to the presence of

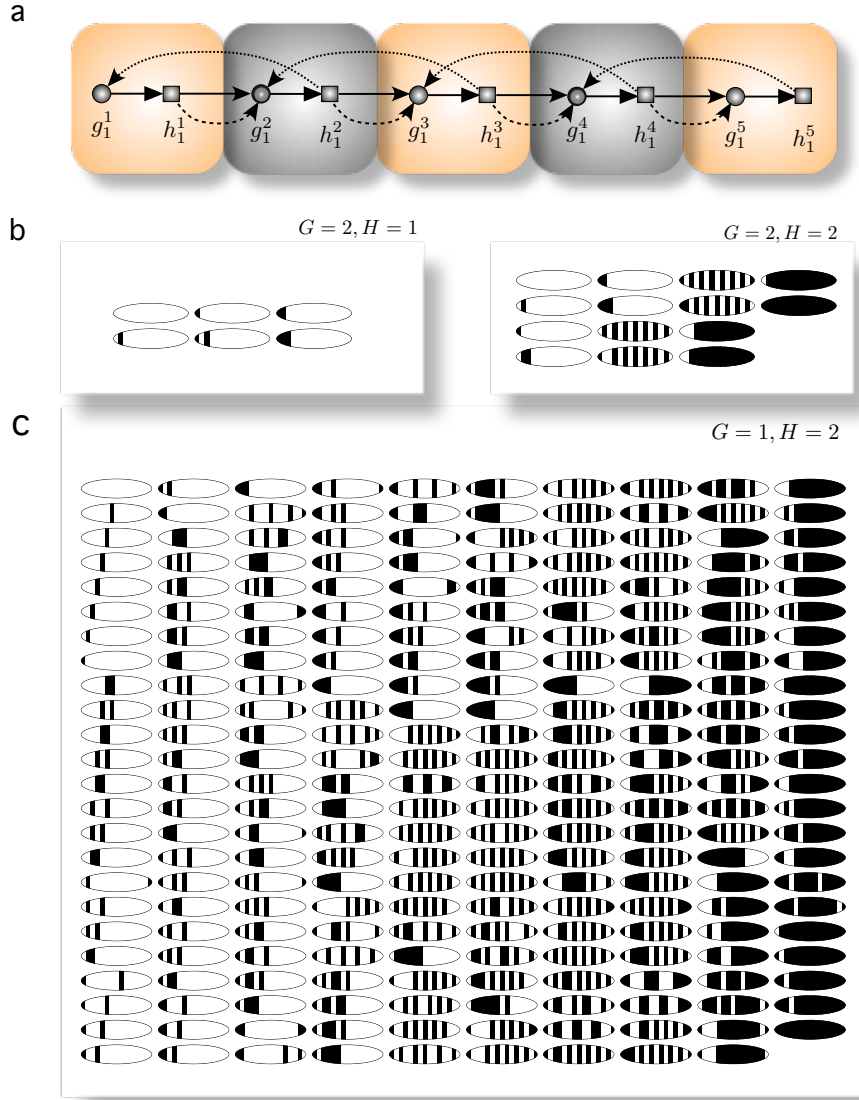


FIG. 9 Transitions from simple to complex (diverse) patterns in a minimal model of gene network model of biological pattern formation. The model considers a population of digital organisms composed by a linear chain of C cells, each carrying the same genetic network (see Box 5). The complexity of this network, measured in terms of the number of genes G associated to internal switches and the number of cell-cell signals H determines the amount of patterns that can be achieved through evolution. In (a) dark and light cells indicate the high and low expression of one of the genes, respectively. In figures (b-c) the complete set of patterns generated in this way is shown for different sets of genes. For $H = 2, G = 1$ or $H = 1, G = 2$ only a few patterns are accessible (b). Once we have $H = G = 2$, all possible patterns can be reached (c).

multiple optima on a morphological landscape. If such optima are easily reached, a diverse range of structures is expected to be obtained. An interesting feature of the space of spatial patterns defined by the gene network model is that it displays neutrality: large, neutral networks percolate sequence space allowing for efficient exploration of the phenotype space. The structure of this pattern forming network space is actually very similar to other found in RNA folding (see Solé et al 2003; Munteanu and Solé 2008). This result tells us something important. As soon as we reach the critical threshold of network complexity, not only multiple patterns become accessible. The intrinsic evolvability of the system is also very high.

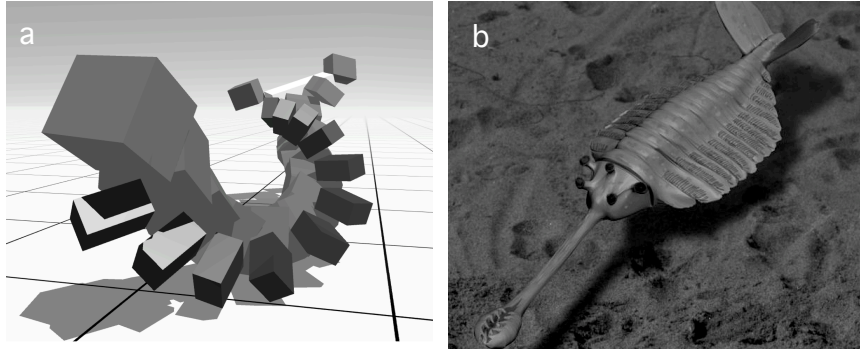


FIG. 10 Virtual creatures (a) can be obtained using physical models of interacting subparts defining a class of artificial life form and evolving under given selection constraints (rendering by Zach Winkler using the Stellar Alchemy program). Although they suggest that we can approach early life forms such as the Cambrian creatures like *Opabinia* (b), they strongly differ in the ways their complexity is generated (rendering by Nobu Tamura).

VII. DISCUSSION AND PROSPECTS

In *silico* models of evolutionary change should be a natural component of our exploration of macroevolutionary patterns and the tempo and mode of evolutionary transitions. Despite their limitations, they offer, along with experimental dynamics using microbial populations (Lenski and Travisano 1994; Elena and Lenski 2003) what no other approach can: an opportunity to recreate potential scenarios and formulate hypothesis about how complexity developed over time. Here we have summarized the outcomes of different models of artificially evolved "organisms" or even communities where different types of transitions in complexity are achieved thanks to the (nonlinear) interplay between genetic and physical -and even ecological- components. Although they are all far from a realistic representation of developmental body plans or true communities, the previous results reveal a great generative potential implicit in the simple rules. In all cases, multicellular complexity undergoes increases or even jumps and some remarkable results can be highlighted. These include, for example, the emergence of some ontogenetic processes resulting from an evolutionary algorithm searching for diverse cell types. Such processes typically incorporate cell-cell interactions that provide the capacity for tissue reorganization and shape changes together with cell differentiation. But even cellular and ecological scales can become related once evolving adhesion provides the exploratory potential for community-level processes to unfold. This connection between such disparate scales provides a novel way of re-considering the problem of hierarchies in evolution (Eldredge 1985; McShea 2001).

Some more sophisticated models have been created which are capable of evolving complex creatures with multiple connected components (figure 10a). These models involve a more or less detailed physical context, both in terms of the elements used to describe the virtual creature and the physics of the environment. Starting from Karl Sims work, these model involve a set of connected components (often parallelepipeds) linked to each other through strings (Sims 1994). The final outcome of this simulations often reminds us of some type of living creature. However, there is an essential difference: in the evolved artificial creatures there is no developmental program at work and thus there is no genotype-phenotype mapping. This is no minor drawback, since developmental programs are the essential component required to properly understand and model evolutionary paths. Over the last years, novel approaches to this problem incorporating some type of morphogenetic rules are being considered (Doursat 2008; Jin 2011).

In the CHIMERA framework, Our artificial creatures are autogenic engineers (Jones 1994): they change their environment mainly via their own physical structures. The success of our model might be due to the complete set of key components that we allow to interact freely. By using space, diverse ecosystems can be build through spatial segregation of subpopulations. By allowing simple components of pattern formation or aggregate generation it is possible to introduce simple forms of cooperative dynamics. By embedding the virtual creatures within an ecosystem where physics plays a role, selection pressures restrict the repertoire of cellular aggregates that can be formed.

Future work should address the potential for generating complex structures perhaps similar to

the Ediacaran fauna and test the role played by both internal and external innovation triggers. The first includes for example the emergence of predators and the resulting arm races, which are known to be a major player in expanding morphological complexity. The second deals with extinctions caused by geological or astronomical shocks which deeply altered communities and whole ecosystems. The aftermath of the extinction provides a unique microscope to see different evolutionary processes in action. Such recovery patterns have been studied both from field data and modeling (Benton and Twitchett 2003; Erwin 1998, 2001; Solé et al 2002; Chen and Benton 2012; Yedid et al 2012) and offer an additional test for studying how organismal and ecological complexity react to stress.

As a final point, it is worth mentioning that another avenue to address the dawn of multicellular systems is provided by synthetic biology (Benner and Sismour 2005; Solé et al. 2007; Cheng and Lu 2012) which is considered by some researchers as the *wet* version of artificial life. By engineering unicellular systems, it is possible to build novel forms of cell-cell communication and thus create (and perhaps re-create) novel forms of multicellular assemblies, able to perform novel functions and even complex computations (Regot et al. 2011; Macia et al 2012; Chuang 2012). Given the potential offered by genetic engineering techniques to alter the logic of cell-cell exchanges, we have a unique opportunity of exploring the landscape of transitions from uni- to multicellular forms of organization.

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